miR-448 in hearts of young dystrophic mice. The latter correlated with overexpression of the Ncf1 gene, encoding the NOX2 regulatory subunit p47phox. Specificity of Ncf1 targeting by miR-448 was confirmed by luciferase reporter assay. To study the effect of miR-448 silencing on molecular, cellular and functional properties of normal hearts, we intravenously injected wild type mice with LNA-NC and LNA-miR-448 inhibitors. qRT-PCR, western blotting, confocal imaging of ROS and intracellular $Ca^{2+}$ signals and echocardiography were employed. Acute inhibition of miR-448 resulted in an increase in Ncf1 expression as well as enhanced ROS production and augmented intracellular $Ca^{2+}$ signaling in isolated cardiomyocytes. In addition, prolong (over one month) inhibition of miR-448 led to the deterioration of cardiac functions and development of dilated cardiomyopathy and arrhythmia. Overall, WT mice with inhibited miR-448 mimicked many features of dystrophic cardiomyopathy. Our data suggest that downregulation of miR-448 relieves inhibition of translational initiation of Ncf1 in dystrophic cardiomyopathy. It results in an increase in Ncf1 expression and consequently in oxidative stress and enhanced $Ca^{2+}$ signaling in dystrophic heart well before cardiac dysfunction becomes evident.

**3921-Pos Board B649**

**Vinculin-Mediated Cytoskeletal Remodeling Modulates Cardiac Morphology and Contractile Function During Ageing**

**Gaurav Kaushik**[1], Alice Spenlehauer[1], Ayla Sessions[2], Danielle Pohl[3], Adriana Trujillo[4], Sanford I. Bernstein[4], Rolf Bodmer[5], Anthony Cammarato[6], Adam J. Engler[1].
[1]Bioengineering, University of California, San Diego, La Jolla, CA, USA, [2]Biomedical Sciences, University of California, San Diego, La Jolla, CA, USA, [3]Iowa State University, Ames, IA, USA, [4]San Diego State University, San Diego, CA, USA, [5]Sanford-Burnham Medical Research Institute, La Jolla, CA, USA, [6]Johns Hopkins University, Baltimore, MD, USA.

Heart failure associated with chronic aging is underscored by altered expression of actin-binding cytoskeletal molecules. How cytoarchitectural remodeling impacts cardiac structure and contractile function remains unclear. We have developed novel biophysical techniques to interrogate subcellular mechanics and cellular function in the genetically tractable, rapidly aging Drosophila, a useful model for mammalian cardiac development. Genotype-dependent, age-related remodeling was a conserved hallmark in wildtype flies signified by decreased systolic and diastolic dimensions between 1 and 5 weeks of age. Remodeling correlated with increased cortical stiffness, particularly at the intercalated disc (ID). Inhibition of actin polymerization reversed the stiffening phenotype at the ID while inhibition of actomyosin crosslinking did not. Age-related remodeling also correlated with preserved contractile function. In contrast, non-remodeled hearts experienced impaired shortening velocities. Cardiac-specific genetic perturbations were employed to determine how altered expression of vinculin (Vcl) affected structure and function independent of aging. Vcl overexpression resulted in increased localization to the ID, decreased diastolic diameter, and increased cortical stiffness at 1 wk of age preferentially at the ID. However, fractional shortening and shortening velocity increased as compared to controls, suggesting that muscle performance was not hindered by Vcl-overexpression despite altered physiology. Vcl-overexpressing hearts treated with cytochalasin D exhibited significant softening at the ID while blebbistatin-treated flies did not, suggesting that Vcl induces stiffening through actin recruitment. In contrast to current models that suggest that intercalated disc remodeling may be maladaptive for age-related cardiac decline, no reduction in systolic function was observed in hearts with Vcl-overexpression. Therefore, Vcl may play a key regulatory role in maintenance of cardiomyocyte structure and function with age. Further studies will investigate the biophysical mechanisms of increased shortening function due to Vcl-mediated remodeling and identify upstream activators of Vcl-upregulation.

**3922-Pos Board B650**

**Novel Locations; Familiar Functions: Obscurin at the Cardiac Intercalated Disc**

**Maegen A. Ackermann**, Nicole A. Perry, Aikaterini Kontrogianni-Konstantopoulos.
University of Maryland, Baltimore, MD, USA.

The intercalated disc (ID) of cardiac muscle embodies a highly ordered, multifunctional network, which is essential for the transmission of electrical stimuli and mechanical force resulting in the synchronous contraction of the heart. Recently, a plethora of proteins have been identified as novel components of the ID. The challenge now lies in their characterization as it relates to the mechanical and electrical coupling of neighbouring cardiomyocytes. Here we focus on the molecular and functional description of one of these novel members, obscurin-90.

Obscurins are a family of proteins expressed in striated muscles where they localize to distinct subdomains, i.e. the sarcoplasmic reticulum, the sarcomeric cytoskeleton, and the sarcolemma, and function in their assembly and integration. Previous studies have shown that transcripts arising from the single OBSCN gene are extensively spliced, resulting in several obscurin isoforms. Complex splicing at its 3' end gives rise to obscurin-90 (obsc90), the isoform that preferentially localizes to the ID. Obsc90 contains tandem RhoGEF and PH motifs, two Ig domains, and a non-modular COOH-terminus with ankyrin-binding sites and ERK phosphorylation cassettes. Using immunofluorescence and immunoelectron microscopy, we show that obsc90 localizes to the ID, and specifically at the outer edge of the transition zone. Consistent with this, biochemical assays demonstrated that obsc90 is in a complex with major ID proteins, including N-cadherin, connexin-43, vinculin, and ankyrinG. Obsc90 is targeted to the ID through the direct binding of its PH domain to membrane lipids, and its functions are likely regulated by phosphorylation of its COOH-terminus. Preliminary evidence indicates that the phosphorylation profile of obsc90 is altered in hypertrophic cardiomyopathy.. Further experiments are underway to examine the function of obsc90 at the ID and its regulation via phosphorylation in health and disease.

**3923-Pos Board B651**

**Acetylation and Phosphorylation Post-Translational Modifications of the CapZ β1 Subunit Regulate FRAP Dynamics Leading to Myocyte Hypertrophy**

**Ying-Hsi Lin**, Chad M. Warren, Brenda Russell.
University of Illinois at Chicago, Chicago, IL, USA.

The mechanism by which more thin filaments are built during cardiac hypertrophy is not fully understood. Very rapid increases the dynamics both actin and the actin capping protein (CapZ) following mechanical flexing suggest that a post-translational regulation is the underlying mechanism. Neonatal rat ventricular myocytes in culture were stimulated to hypertrophy by a neurohormone (10μM phenylephrine, PE, for 24 hr). CapZ dynamics were analyzed by fluorescence recovery after photobleaching (FRAP) using CapZβ1-GFP. After PE treatment, CapZ dynamics increased above resting controls by ~3.17 fold ($p=0.0004$). Post-translational modifications of CapZ were analyzed by 2D gel electrophoresis. After PE treatment, 2D spots of CapZβ1-GFP have an increased negative shift, suggesting that post-translational modification of CapZ is up regulated. To identify the types of post-translational modifications, 2D western blotting and mass spectrometry (MS) was applied. Increased post-translational spots included the acetylation of K199 and phosphorylation of S204, which are both close to the actin-binding region of CapZ. To test whether CapZ acetylation was mediated by HDAC3, located at the Z-disc of myocytes, the class I HDAC inhibitor (5μM trichostatin A / 5hr) and HDAC3 activator (10μM theophylline / 24hr) were applied. CapZ dynamics with trichostatin A increased by four-fold ($p=0.01$), and the effect of PE on CapZ dynamics was blunted by theophylline ($p=0.09$). Thus, the increased sarcomere remodeling during cardiac hypertrophy may be induced via altered acetylation of CapZ, which is mediated by HDAC3. In addition to MS identification of phosphorylation sites, post-translational modifications were reduced by dominant negative PKCε, suggesting a regulatory role. Together the acetyl and phospho posttranslational modifications of CapZ reduce the capping property and may increase thin filament assembly. NIH HL62426 (BR) and AHA 12PRE12050371 (Y-H L).

**3924-Pos Board B652**

**Actin Carbonylation is Higher in Human Hypertrophic Cardiomyopathy Due to MYH7 Mutations**

**Rosalie Witjas-Paalberends**[1], Marcella Canton[2], Michelle Michels[3], Carolyn Ho[4], Corrado Poggesi[5], Fabio di Lisa[2], Jolanda van der Velden[1,6].
[1]VU University Medical Center, Amsterdam, Netherlands, [2]University of Padova, Padova, Italy, [3]Erasmus Medical Center, Rotterdam, Netherlands, [4]Brigham and Women's Hospital, Boston, MA, USA, [5]University of Florence, Florence, Italy, [6]ICIN-Netherlands Heart Institute, Utrecht, Netherlands.

**Introduction:** Hypertrophic cardiomyopathy(HCM) is frequently caused by mutations in genes encoding sarcomeric proteins. Decreased force development was found in human HCM due to mutations in genes encoding myosin binding protein-C(MYBPC3), myosin heavy chain(MYH7) and in sarcomere mutation-negative HCM(HCMsmn). This is partially caused by the mutation (especially MYH7 mutations), increased cell size and reduced myofibrillar density. However, 10% force reduction could not be explained by the mutation or cellular remodeling. In end-stage human heart failure increased actin-carbonylation, induced by oxidative stress, negatively correlated with myocardial function. Therefore, we investigated whether actin is carbonylated in human HCM.

**Methods and results:** Left ventricular tissue of human HCMsmn, MYBPC3-mut and MYH7mut patients was obtained. Non-failing donors were used as control. Actin-carbonylation was analyzed using Oxyblot. Carbonyl groups of myofilament proteins were derivatized by 2,4-dinitrophenyl-hydrazine(DNPH). Protein lysates were processed via SDS-page, western blot